AMINO ACID CONTENT OF COMMERCIAL BROILER RATIONS

by

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INTRODUCTION AND REVIEW OF LITERATURE

Meat scrap, fish meal and dried milk constitute the greater portion of the animal protein of commercial broiler rations. In recent years available protein of animal origin has been greatly reduced in quantity, possibly in quality, and in some instances has become unprocurable. As a result, producers of commercial rations in many cases have had to replace a large part of the animal protein with plant protein. This substitution of plant protein may lead to a deficiency of certain of the essential amino acids such as methionine, tryptophane and lysine. A ration deficient in any of these essential amino acids would restrict growth in the chick.

Studies have been made of the essential amino acids required by the chick and reported results are in agreement. Almquist and Grau (1944) and Almquist (1945) reported the requirement of amino acids for the chick to be as shown in Table 1. Hegsted (1944) reported the same amino acids to be essential. Luckey et al. (1947) reported diets containing

Table 1. Required amino acids for the chick in per cent of total ration.

Amino acid	Per cent	Amino acid	Per cent
Glycine	0.8-1.00	l-Leucine	1.50
l-Arginine	0.90	l-Phenylalanine	1.00
l-Lysine	0.90	l-Threonine	0.5-1.00
l-Tryptophane	0.25	l-Valine	1.00
l-Histidine	0.15	dl-Methionine [1]	0.50
l-Isoleucine	1.00	l-Cystine	0.40

[1] 0.9 per cent methionine is required in the absence of cystine.

some of the nonessential amino acids are superior to diets containing the 11 now recognized as essential for the chick.

As previously stated, methionine, tryptophane and lysine are the amino acids most likely to be low in a commercial broiler ration. There are three recognized methods of amino acid assay; namely, (a) feeding trials, (b) microbiological methods, and (c) chemical methods. An amino acid deficiency will be indicated by a decrease in the growth rate of chicks or of other experimental animals receiving the experimental ration. Microbiological and chemical assays are means of determining the exact amount of amino acid present in the ration. Any deficiency of amino acid may then be determined by comparing the amount found to be present with the amount required to give maximum or satisfactory growth. Comparison of results obtained by feeding trials and microbiological determinations will be emphasized herein.

Adequacy of the rations with respect to amino acids essential for the chick was determined by feeding each ration with and without a commercial amino acid supplement or crystalline methionine.

Microbiological assay methods for amino acid determinations have been developed by Green and Black (1944) Dunn et al. (1944, 1944, 1945) Stokes et al. (1945) Riesen et al. (1946) Lyman et al. (1946, 1946, 1947) and others, based on measurement of lactic acid produced during growth of the organism. These methods of assay have been perfected to give the amino acid content with accuracy within five per cent; Dunn et al. (1944 p. 715).

The amounts and distribution of the amino acids in feedstuff proteins are primary factors determining the value of the proteins in the nutrition of the chick. The National Research Council has made recommendations as to the amounts of methionine, cystine, lysine and tryptophane that should be contained in a ration for growing chicks. The purpose of this experiment is to determine whether commercial broiler rations commonly used in Kansas meet these requirements.

PROCEDURE

Microbiological Analyses

Four widely advertised broiler rations were analyzed. The microbiological method of assay consists in use of a medium complete, except for the amino acid to be determined. Different amounts of hydrolyzed material to be assayed were added to tubes of this medium, which was innoculated with a suitable organism. After a suitable period of incubation, the acid formed was titrated with standard sodium hydroxide. A standard curve is obtained by running a set of tubes containing various amounts of the amino acid to be assayed and plotting the volume of sodium hydroxide required to titrate the acid formed against the amount of amino acid used (Figs. 1 and 2). The amino acid value of any assay tube is obtained by reading the amino acid value from the standard curve corresponding to the volume of sodium hydroxide used for titration.

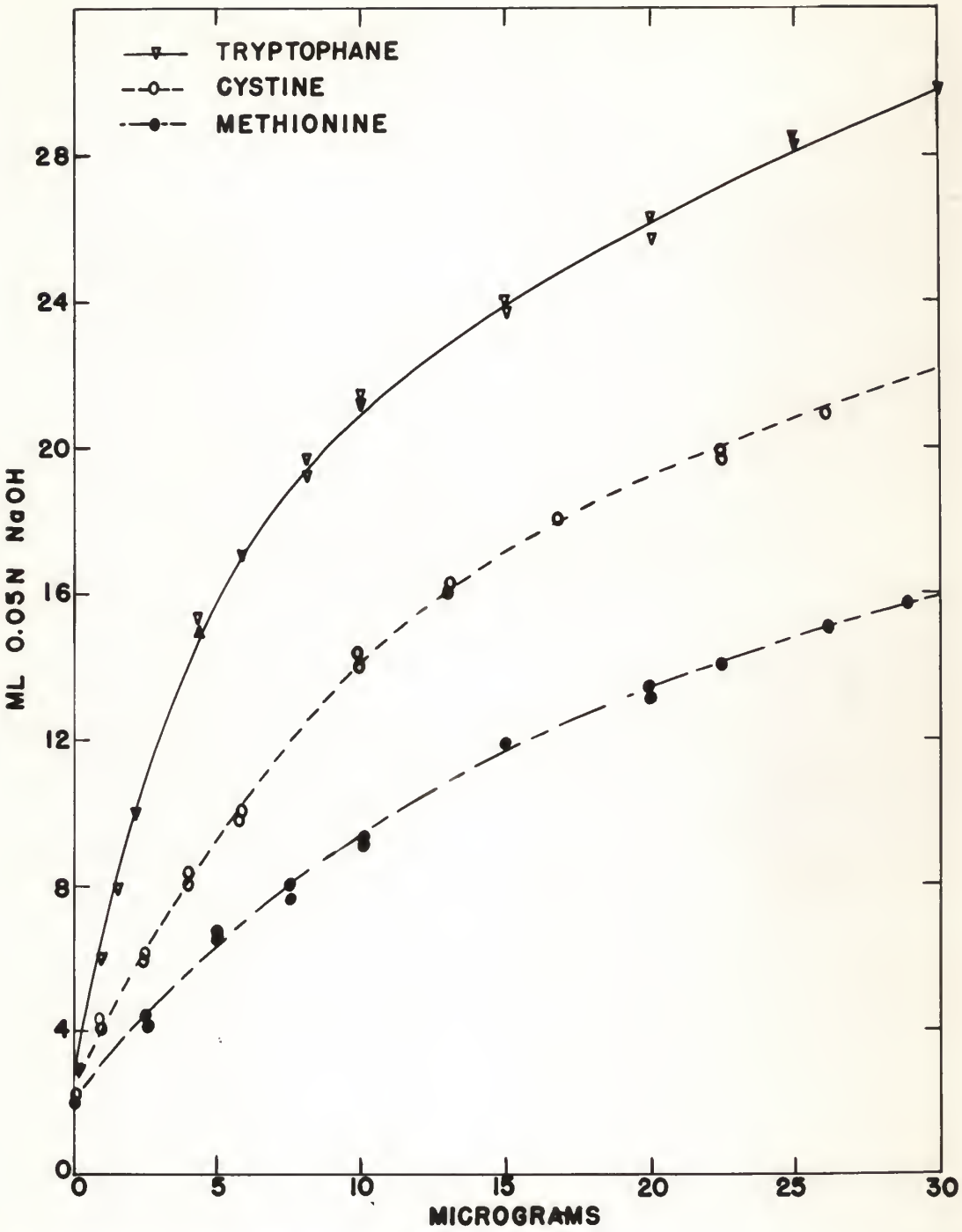

FIG. 1. STANDARD AMINO ACID CURVES.

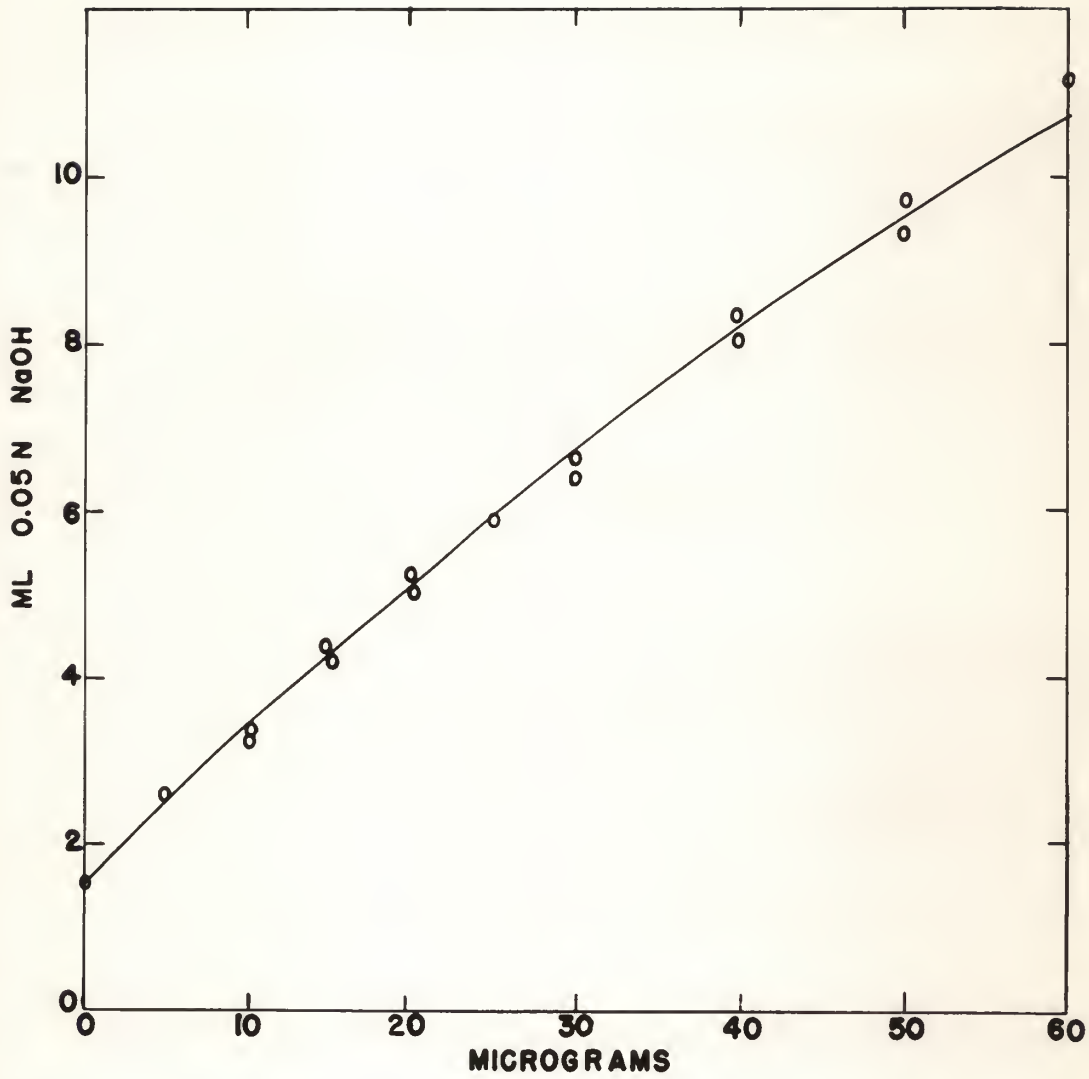

FIG. 2. STANDARD LYSINE CURVE.

In this work standard curves were obtained by using duplicate amounts of a standard solution ranging from 0-50 micrograms of dl-methionine, 0-10 micrograms of dl-tryptophane, 0-100 micrograms of l-lysine and 0-50 micrograms of l-cystine respectively.

Samples for the determination of lysine, methionine and cystine were prepared by hydrolyzing one g samples with 20 ml of two normal hydrochloric acid for 8-10 hours at 15 pounds pressure. The hyrolysates were cooled and filtered; the pH was adjusted to 6.8 with sodium hydroxide, and the samples were diluted to 200 ml volume.

For the determination of tryptophane a method, developed by Green and Black (1944), was followed. Five-tenths-gram samples were mixed with 4.2 g anhydrous barium hydroxide and 8 ml of water in 25 ml Erlenmeyer flasks, hydrolyzed for 7 hours at 15 pounds pressure, cooled, and hydrolysates were transferred to 250 ml centrifuge tubes. Ten normal sulfuric acid was added until pH of 4 was reached using methyl orange as indicator. The mix was diluted to 100.0 ml, heated in a water bath to 50-60 degrees centigrade, covered and centrifuged. The separated liquid was cooled and extracted three times with 100 ml portions of diethyl ether followed by 30 ml toluene. The pH was adjusted to 6.8 and the volume to 200 ml.

One-gram samples of hydrolyzed broiler ration diluted to 200 ml were used for analysis. Aliquouts containing from 1-3 mg of the hydrolyzed ration were used for determination of

methionine; 1-3.5 mg for lysine, and 4-4.5 mg for cystine. For the determination of tryptophane 0.3-1 ml of the alkaline hydrolysate, containing 0.5 g of ration in 200 ml, was used, which is equivalent to 0.75-2.5 mg of ration.

Determination of the same amino acids was made on the commercial amino acid mixture which did not require hydrolysis. This commercial amino acid mixture was the amino acid residues of hydrolyzed wheat gluten after removal of a large portion of the glutamic acid in the form of its sodium salt.

The amino acids required in the assay medium were supplied, for the most part, by a relatively simple peptone preparation. Based on the preferential oxidation method of Toennies and Callan (1939) and Lyman et al. (1946), a method has been developed for removing methionine, cystine, tryptophane and tyrosine from peptone preparations by hydrogen peroxide oxidation. The method of preparing hydrogen peroxide-treated peptone according to Lyman et al. (1946) is as follows: 50 g of Bacto-peptone are dissolved in 250 ml of water and 250 ml of 2 N HCl are added after the peptone is completely dissolved; 0.025 mole of reagent grade hydrogen peroxide (2.8 g of 30 per cent hydrogen peroxide) is added and the solution allowed to stand over night at room temperature. The material then is steamed for 30 minutes at atmospheric pressure, stirred while hot, cooled, neutralized with sodium hydroxide, and steamed again for 1 hour. The preparation is ready for use after diluting to a final volume of 1 liter. The preparation is acidified and may be used even after several weeks if stored in the refrigerator.

The basic media for cystine, methionine, and tryptophane determinations, a modification of that of Lyman et al. (1946 p. 161-171), is given in Table 2. Amounts of several of the vitamins were increased.

Table 2. Medium[1] for determination of methionine, cystine and tryptophane.

Ingredient	Amount	Ingredient	Amount
H_2O_2-treated peptone	150 ml or(7.5) g	Thiamin	0.1 g
		Pyridoxine	0.1 g
Glucose	20.0 g	Calcium pantothenate	0.1 g
Sodium acetate	12.0 g	Riboflavin	0.1 g
l-Tryptophane[2]	0.1 g	Niacin	0.2 g
l-Cystine[2]	0.1 g	Pyridoxamine	0.1 g
dl-Methionine[2]	0.1 g	Biotin	5.0 µg
dl-Tyrosine	0.1 g	Para-amino benzoic acid	0.2 µg
Adenine Sulfate	20.0 mg	Folic acid	10.0 µg
Guanine	20.0 mg	Salt solution A[3]	5.0 ml
Uracil	20.0 mg	Salt solution B[4]	5.0 ml
Xanthine	20.0 mg	Salt solution C[5]	2.5 ml
		Neutralize and dilute to 500 ml.	

[1]Medium for 100 cultures of 10 ml final volume (5 ml of the above medium per culture).
[2]Amino acid to be assayed is left out of the medium.
[3]Salt solution A - K_2HPO_4 25 g, KH_2PO_4 25 g, water 250 ml.
[4]Salt solution B - $MgSO_4.7H_2O$ 10.0 g, NaCl 0.5 g, $MnSO_4.4H_2O$ 0.5 g, water 250 ml.
[5]Salt solution C - $FeSO_4.7H_2O$ 0.5 g, water 250 ml.

The medium shown in Table 2 was used by Lyman et al. (1946 p. 161-171) to determine methionine. It is more convenient and gives more consistent results than an amino acid mixture as is required for the determination of lysine. Leuconostoc mesenteroides (PDGO) was chosen as the organism for the

determinations because all four of the amino acids determined are required by this organism, and growth of this organism is not influenced by other conditions such as salt concentration, etc. The incubation period was 60 hours in all cases.

For the determination of lysine, amino acids shown in Table 3 were substituted for the hydrogen peroxide-treated peptone in the foregoing basal medium since lysine is not oxidized by hydrogen peroxide. This amino acid mixture is based upon the work of Dunn et al. (1944, p. 703-713) on the requirements of Leuconostoc mesenteroides for maximum growth. The remainder of the basal medium remains the same. This medium was found to give satisfactory results.

Table 3. Amino acids[1] for lysine determination.

Amino Acid	Amount	Amino acid	Amount
dl-Alanine	0.50 g	dl-Phenylalanine	0.10 g
l-Aspargine	1.30 g	l-Arginine·HCl	0.10 g
dl-Threonine	0.25 g	Glycine	0.10 g
l-Glutamic acid	0.20 g	dl-Serine	0.10 g
dl-Valine	0.10 g	l-Histidine	0.05 g
dl-Isoleucine	0.10 g	l-Proline	0.05 g
dl-Leucine	0.10 g		

[1] Medium for 100 cultures of 10 ml final volume (5 ml of the above medium per culture).

Leuconostoc mesenteroides was the only organism used for which results were tabulated. This organism was kept on a carrying media consisting of glucose 1.0 g, Bacto-peptone 0.5 g, anhydrous sodium acetate 0.6 g, agar agar 1.5 g, salt solution

A 5 ml, salt solution B 5 ml and salt solution C 2.5 ml. The pH was adjusted to 6.8 and the volume was made up to 100.0 ml. Tubes containing 10 ml of this media, sterilized, were used for carrying the organism. The organism was transferred at least bi-weekly. For use in the determinations, a fresh inoculum was prepared by inoculating tubes of the above media containing no agar agar with the test organism, and incubating for 24 hours at 37 degrees centigrade. The organisms were centrifuged, washed three times with 0.9 per cent saline solution, centrifuged after each washing, and then suspended in 20 ml of 0.9 per cent saline for inoculation of the assay tubes.

Feeding Trials

A preliminary trial was first conducted. A widely advertised commercial broiler ration, ration A, was used as the basal ration. Fifty day-old-hybrid-sexed chicks were divided by random selection, wing banded and placed in three separate lots in a constant temperature room. The constant temperature room was maintained at 31 degrees centigrade throughout the experiment. Cages were of wire gauze and false bottoms to make for easy cleaning. The chicks were fed and watered ad libitum for a period of six weeks and determinations were made of weight gains weekly.

Group I received only the commercial broiler ration. Group II received the same commercial broiler ration supplemented with 0.25 per cent of the commercially prepared amino

acid mixture. Group III received the same commercial broiler ration supplemented with 0.1 per cent crystalline methionine.

A second feeding trial was conducted in like manner. Three other widely advertised commercial broiler rations were fed ad libitum unsupplemented and supplemented with the amino acid mixture to lots containing 20 day-old New Hampshire chicks and growth determined weekly for six weeks by weight gains. Groups IV, VI and VIII received the unsupplemented commercial broiler rations, and groups V, VII and IX received the supplemented commercial broiler rations.

RESULTS

Microbiological Analyses

At least two series of analyses were made for each amino acid determined and an average of the two analyses taken as the amount of each amino acid present in the ration. It was found that variations between different series of analyses on the same hydrolysate were as great as variations between different hydrolysates. Quantitative techniques were adhered to throughout the study.

After the incubation period, standard and assay tubes were titrated with 0.05 normal sodium hydroxide. Standard curves were plotted and the concentrations of amino acids were determined by reference to the standard curves (Figs. 1 and 2).

Typical data, titration values, calculated amounts of amino acid in each tube, and per cent of amino acid in the sample are shown in Table 4.

Table 4. Determination of lysine[1] with Leuconostoc mesenteroides.

Aliquot	ml 0.05 N NaOH		Lysine equiv. (micrograms)		Lysine (per cent)	
	I	II	I	II	I	II
.2	3.5	3.6	10.5	10.6	1.05	1.06
.3	4.3	4.2	15.2	15.0	1.01	1.01
.4	5.0	5.0	19.5	19.5	0.98	0.98
.5	6.1	6.1	26.0	26.0	1.04	1.04
.6	6.5	6.5	28.5	28.5	0.95	0.95
.7	7.2	7.3	33.0	33.5	0.95	0.95

[1] 1 g sample in 200 ml dilution.
I and II refer to different series of assay.

Microbiological analysis of the commercial amino acid mixture used for supplementing the rations showed the following amounts of the amino acids determined: methionine 2.14 per cent, cystine 2.3 per cent, lysine 0.393 per cent, and tryptophane 0.0 per cent. Similar analyses of the broiler rations, unsupplemented and supplemented, are shown in Table 5. The amino acid content of the unsupplemented broiler rations vary somewhat. Values for methionine range from 0.248 per cent for ration A to 0.302 per cent for ration D. The variation in cystine ranges from 0.136 per cent to 0.146 per cent. Ration B was found to contain the most lysine, having a value of 1.156

Table 5. Analysis of the broiler rations.

Ration	Methionine	Cystine (per cent[1])	Lysine	Tryptophane
A	0.248	0.136	0.974	0.221
B	0.266	0.139	1.156	0.234
B & Supp.	0.278	0.147	1.163	0.234[2]
C	0.281	0.146	1.044	0.240[2]
C & Supp.	0.295	0.154	1.051	0.235[2]
D	0.302	0.137	1.028	0.197
D & Supp.	0.313	0.144	1.035	0.200

[1]Per cent of total ration.
[2]Apparent discrepancy due to fact supplement contained no tryptophane and accuracy of analysis is five per cent. Dunn et al. (1944).

per cent as compared with ration A, containing 0.974 per cent of this amino acid. Tryptophane content of ration C was greatest, having a value of 0.240 per cent as compared with ration D which contained only 0.197 per cent. No analysis was made on the supplemented ration A since it was a preliminary feeding trial.

Feeding Trials

Results of the first feeding trial are shown by growth curves in Fig. 3. Using commercial feed A as the basal ration, both supplemented groups show significantly (P 0.01) better growth at five weeks of age over those fed ration A alone. The best growth for the first two weeks was attained by those chicks supplemented with the amino acid mixture. After the third week, the growth curves indicate slightly better growth of the chicks supplemented with 0.1 per cent crystalline dl-methionine.

Results of the second feeding trial are shown by growth curves in Figs. 4, 5 and 6. In the case of commercial feed B, the supplemented ration produced slightly better growth for the same period of time than did the unsupplemented ration. There was little difference in growth rate of the groups of chicks on commercial feed C, unsupplemented and supplemented.

The unsupplemented commercial feed D produced better average growth than did the supplemented ration. The chicks of the supplemented group did not consume as much feed as the unsupplemented group, and severe diarrhea was prevalent throughout the group for the first three weeks of the feeding trial. There was considerable variation in the weights of the chicks of this group.

Of the three unsupplemented commercial broiler rations used in the second feeding trial, ration D gave better growth than did ration C. Ration B gave slightly better growth than did ration C but growth was not as good as on ration D. Ration A of the first feeding trial can not be compared with rations B, C and D of the second feeding trial because the first trial was conducted at a different time and on a different breed of chicks.

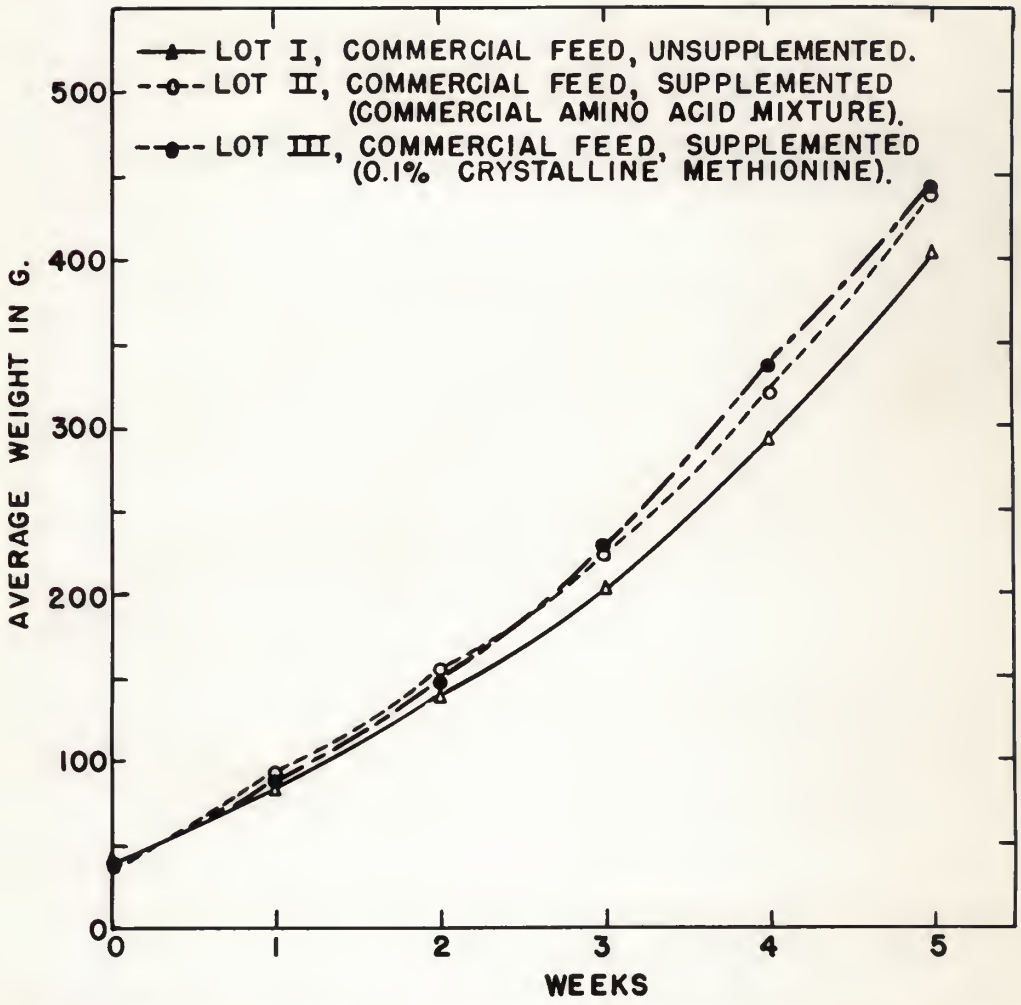

FIG. 3. GROWTH CURVES FOR CHICKS ON COMMERCIAL BROILER RATION A UNSUPPLEMENTED AND SUPPLEMENTED WITH AMINO ACIDS.

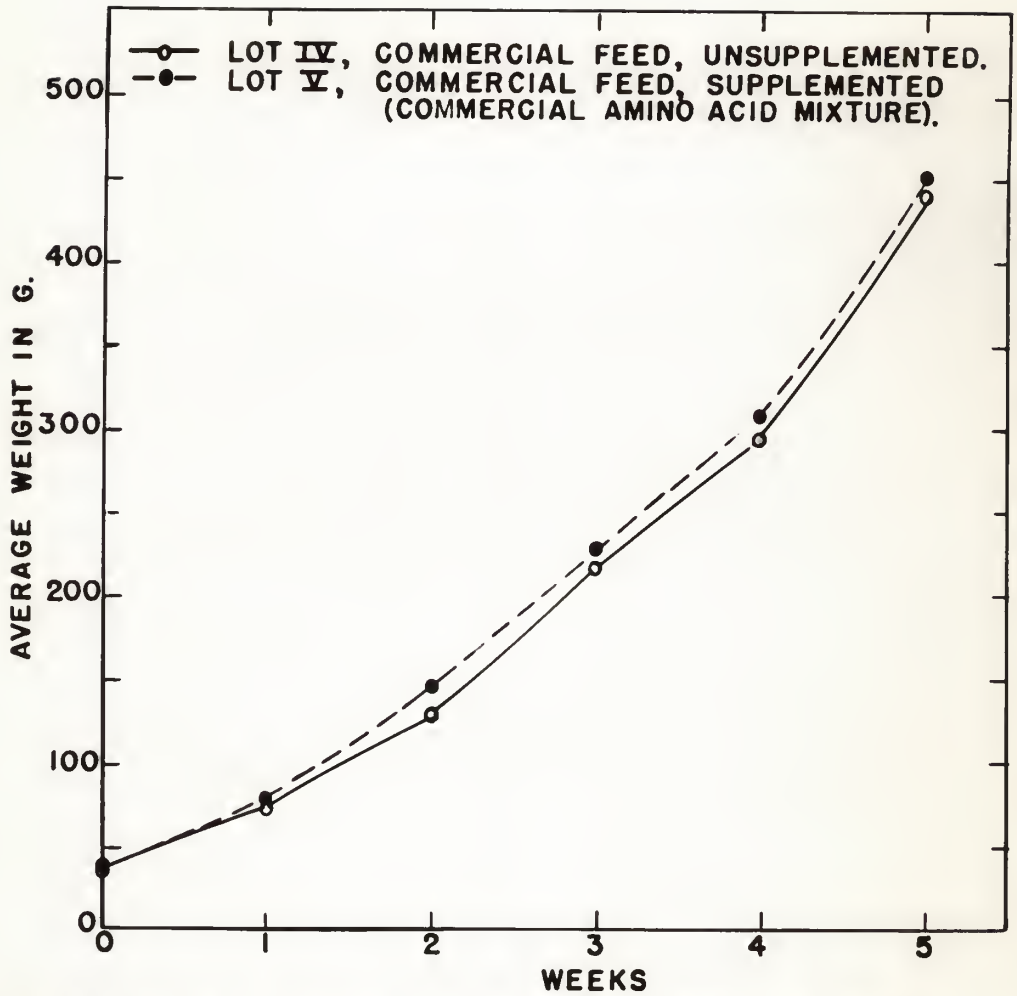

FIG. 4. GROWTH CURVES FOR CHICKS ON COMMERCIAL BROILER RATION B UNSUPPLEMENTED AND SUPPLEMENTED WITH AMINO ACIDS.

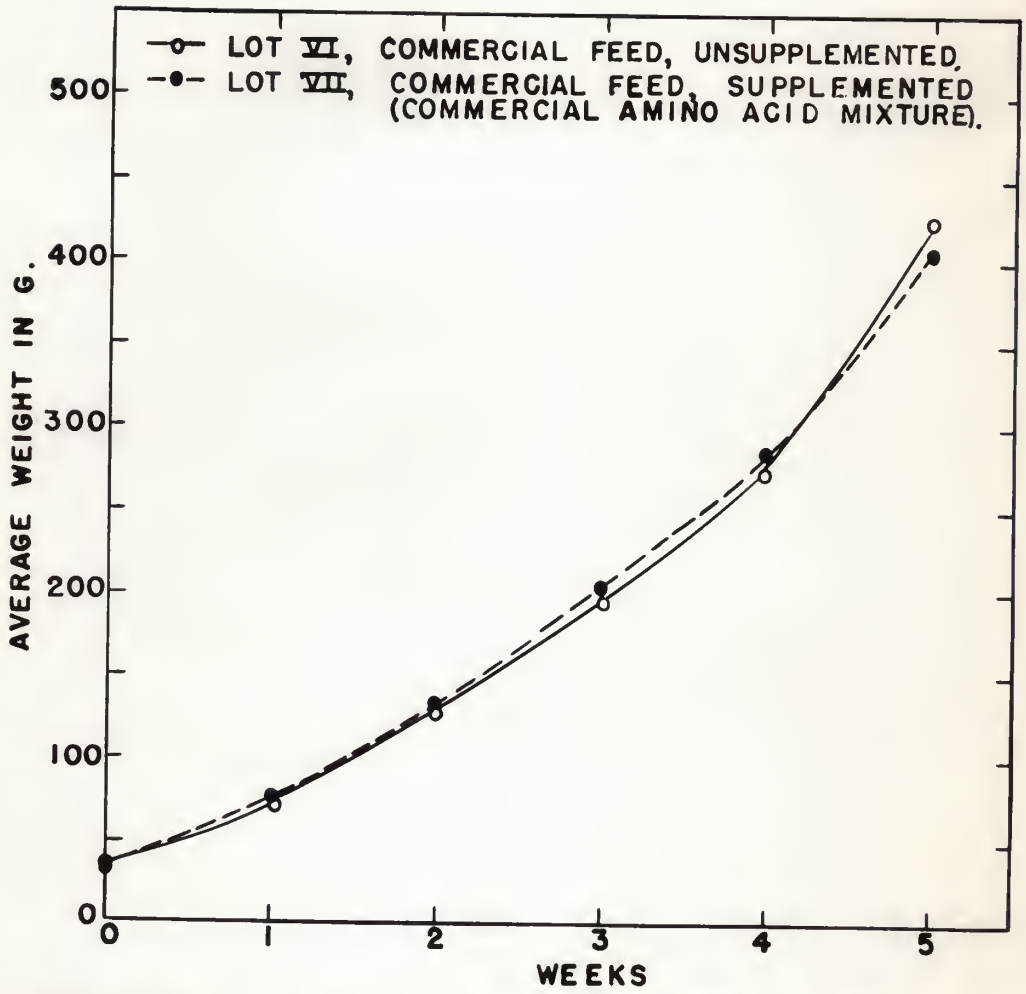

FIG. 5. GROWTH CURVES FOR CHICKS ON COMMERCIAL BROILER RATION C UNSUPPLEMENTED AND SUPPLEMENTED WITH AMINO ACIDS.

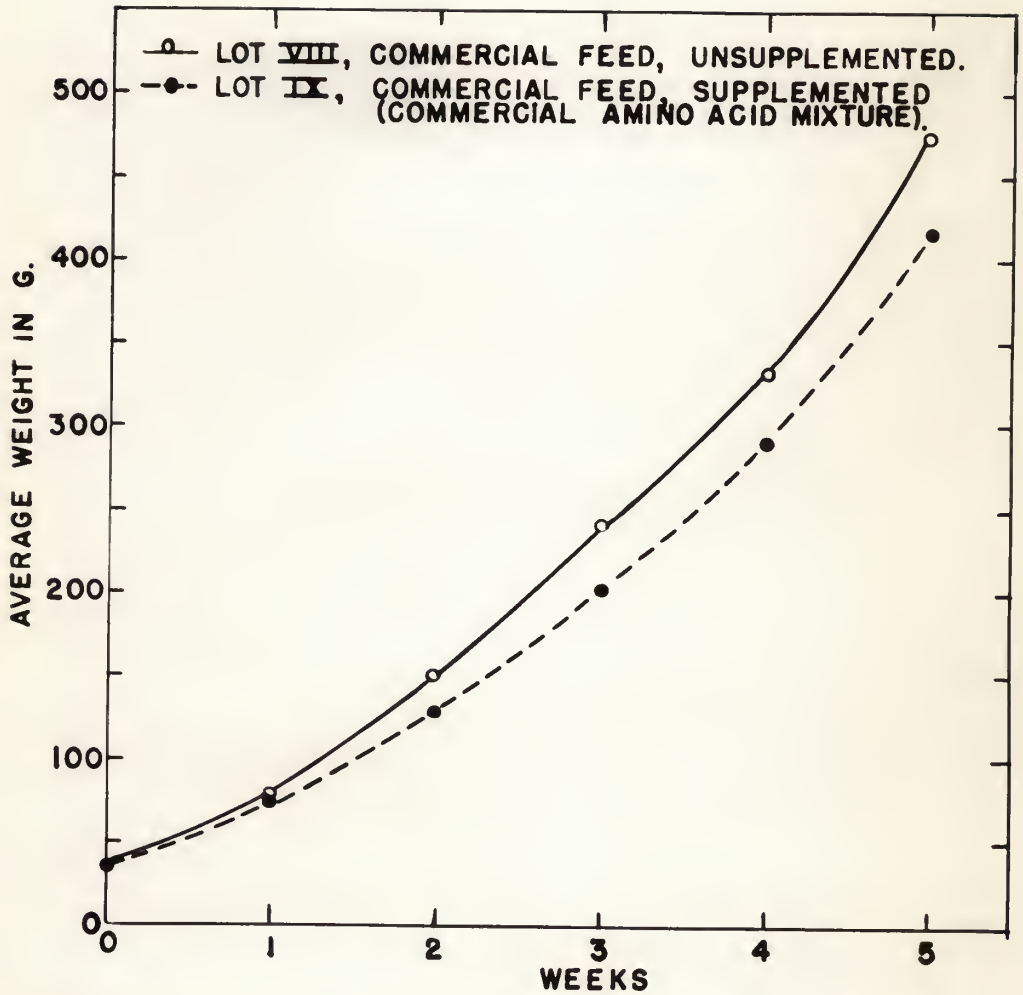

FIG. 6. GROWTH CURVES FOR CHICKS ON COMMERCIAL BROILER RATION D UNSUPPLEMENTED AND SUPPLEMENTED WITH AMINO ACIDS.

DISCUSSION OF RESULTS

Values obtained by analysis of the commercial broiler rations for methionine, cystine, and tryptophane by the microbiological method are considerably lower than recommended values given by Almquist and Grau (1944) and by the National Research Council (1944) for growing chicks. Lysine content was found to meet their recommendations. The methionine requirement is dependent upon the cystine present. If the amount of cystine is deficient the level of methionine should be increased. According to analysis, the total amounts of these amino acids for each of the rations was found to be approximately one half of the recommended value.

Comparable increase in growth rate was obtained by the addition of 0.1 g crystalline dl-methionine per 100 g of ration as by the addition of 0.25 g amino acid mixture per 100 g of ration. This indicates that there was a sub-optimum of methionine and cystine in the ration. Since the amino acid mixture which increased the methionine only by 0.0054 per cent and cystine only by 0.0058 per cent produced as much increase in growth as the addition of 0.1 per cent crystalline dl-methionine, it is possible that there is a deficiency of some undetermined amino acid in addition to methionine.

Results of the two methods of testing the ration (microbiological and chick growth) are in agreement. The ration found lowest in methionine by bacteriological analysis was

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supplemented by the addition of the amino acid mixture as shown by a significant increase in growth. The supplementation of two other rations gave slightly better growth results over the same growth period. For ration D, the experimental group of chicks grew better on the unsupplemented ration than did those on the ration supplemented with the commercial amino acid mixture. This ration was found to have the highest methionine content, which seems to be the limiting growth factor, of the four rations. This decreased growth on the supplemented ration is not believed to be caused by the addition of 0.25 per cent amino acid mixture. A marked degree of diarrhea and a lower feed consumption than for chicks of any other group was observed during the early growth period. Individual variance of weights within this group were considerable at the termination of the feeding trial. It has not been determined to what degree the foregoing observations might be related to the decreased growth observed for chicks receiving ration D plus amino acid supplement.

The results of the microbiological determinations and growth experiments would indicate that some commonly advertised commercial broiler rations in Kansas may contain a sub-optimum of methionine, cystine, tryptophane and possibly other amino acids for growing chicks. Further investigation of this problem would be in order.

SUMMARY

1. The adequacy of the amino acids methionine, cystine, lysine and tryptophane in four widely advertised commercial broiler rations was determined by subjecting the rations to microbiological assay and feeding trials with growing chicks. In the feeding trials the rations were fed unsupplemented and supplemented with a commercial amino acid supplement or cystalline dl-methionine.

2. Microbiological results indicate methionine, cystine and tryptophane content of the broiler rations to be sub-optimum. Lysine content was found to meet the recommended level for growing chicks.

3. Growth experiments support results obtained by microbiological analyses. Addition of crystalline dl-methionine to one of the rations significantly improved the growth rate of chicks to five weeks of age. Supplementation with the amino acid mixture improved growth on two of the four rations.

4. Indications are that some essential amino acid other than those determined may be sub-optimum.

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The author wishes to take this opportunity to express his appreciation to Dr. J. S. Hughes, Acting Head of the Department of Chemistry, for assistance in selecting this problem and for giving valuable advice in connection with the feeding trials. A special note of appreciation is due Dr. R. E. Guerrant of the Department of Chemistry whose constant assistance and encouragement during microbiological determinations contributed materially to the completion of this problem. Recognition is also due World Products Corporation for providing the commercial amino acid mixture and help to defray the expense of the feeding trials.

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